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PTO-103P
(Rev. 8-95) PROVISIONAL APPLICATION
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APPLICATION NUMBER	FILING DATE	FIL FEE REC'D	ATTORNEY DOCKET NO.	DRWGS
60/100,914	09/17/98	$75.00	110.00810160	15

ANN M MUETING
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POST OFFICE BOX 581415
MINNEAPOLIS MN 55458-1415

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TITLE
DEVICE FOR MEASURING METALS

DATA ENTRY BY: YON, LOWUAN TEAM: 05 DATE: 04/07/99

(see reverse)

DEVICES FOR MEASURING METALS

Field of the Invention

The present invention provides devices for measuring metals, particularly toxic metals such as mercury.

Background of the Invention

Metal contamination, particularly mercury contamination, continues to be a public health and environmental problem. Conventional chemical detection techniques include atomic absorption spectrophotometery, ion chromatography, gas chromatography, mass spectrometry, as well as cold-vapor atomic absorption or cold-vapor atomic fluorescence spectroscopy. At least some of these techniques can be highly sensitive but complex to perform and expensive in terms of equipment and training. Furthermore, these techniques must typically be conducted in the laboratory. Plus, these techniques do not always reflect the true biological availability of toxic metals in a system.

Microorganisms that quantitatively detect toxins in the environment offer a less expensive alternative to conventional methods. For example, microbial biosensors aimed at measuring the bioavailability of mercury have been developed as an alternative to chemical or physical analysis. U.S. Patent No. 5,612,184 (Rosson) discloses a device for the detection of mercury in water using an aqueous suspension of recombinant biosensory microorganism cells containing a *lux* bioluminescence gene. The cells are bioluminescent in the presence of Hg^{2+} ions and/or monomethyl mercury. The resultant bioluminescence can be detected using a variety of means, e.g., photographic film, photomultiplier, photodiode, or scintillation counter. However, this patent only discloses the use of such a suspended cell biosensor for the detection of mercury in water. Furthermore, suspended cell biosensors are limited because of handling difficulties and short useful life of the cell stock solution.

Immobilization of cells for the biodetection of contaminants in aqueous environments offers advantages over the use of suspended cell systems. Immobilized cells are easy to handle, can remain viable for long periods of time, and show excellent plasmid retention. However, immobilization methods for use in biosensors have focused on reusable detection methods where the immobilization cells are used repeatedly. In such methods, control over immobilized cell stability and cell outgrowth become considerable problems together with slow biosensor response times.

Thus, a need exists for a detection device, particularly one that is portable, inexpensive, and easily standardized, for use on samples such as solids, semisolids, and liquids, particularly tissue samples, that can be used to quickly detect, and preferably determine the true level of, toxic mercury or other metals.

Summary of the Invention

The present invention provide a device for determining the presence of a metal in a sample comprising a cell-containing indicator coating supported on a substrate, wherein, upon contact with the metal, the cells produce a response and emit a signal. Preferably, the cell-containing indicator coating comprises cells, such as bacterial cells, immobilized in one or more layers of a polymeric material. Preferably, the cells are genetically engineered to produce a response, such as luminescence, to the metal of interest. In certain embodiments, the substrate is capable of detecting the signal. In such embodiments, the substrate is a photosensitive film or a light-sensitive electronic chip, for example.

In a preferred embodiment, the present invention provides a device for determining the presence of mercury in a sample comprising a cell-containing indicator coating supported on a substrate, wherein, upon contact with the mercury, the cells produce a response and emit a signal that is detected by the substrate.

The present invention also provides a method of determining the presence of a metal in a sample. The method comprises contacting the sample with a device comprising a cell-containing indicator coating supported on a substrate, wherein, upon contact with the metal, the cells produce a response and emit a signal; and detecting the signal.

Brief Description of the Drawings

Figure 1A is a cross-sectional schematic of one embodiment of a device according to the present invention using a photosensitive film as the supporting substrate.

Figure 1B is a bottom view of the device of Figure 1A showing the photosensitive film.

Figure 2A is a cross-sectional schematic of an alternative embodiment of a device according to the present invention using a light-sensitive electronic chip as the supporting substrate.

Figure 2B is a bottom view of the device of Figure 2A showing the light-sensitive electronic chip.

Figure 3 is a cross-sectional schematic of an alternative embodiment of a device according to the present invention using a solid light-proof or light impenetrable backing as the supporting substrate.

Figure 4 is a cross-sectional schematic of an alternative embodiment of a device according to the present invention that is used to penetrate a solid sample such as fish tissue.

Figure 5 is a cross-sectional schematic of an alternative embodiment of a device according to the present invention that includes a cavity for placement of a solid sample, such as fish tissue.

Figure 6 is a cross-sectional schematic of an alternative embodiment of a device according to the present invention that includes a cavity for placement of a solid sample containing a fluid, such as an absorbent pad containing fluids from fish tissue.

Figure 7A is a schematic of an alternatively embodiment of a device according to the present invention that can be used to penetrate through fish scales, skin, and tissue and remove a core sample of tissue.

Figure 7B shows the device of Figure 7A penetrating fish scales, skin, and tissue.

Figure 8A is a schematic of an alternative embodiment of a device according to the present invention that can be used to penetrate through fish scales, skin, and tissue.

Figure 8B shows the device of Figure 7A penetrating fish scales, skin, and tissue.

Figure 9A is a cross-sectional schematic of an alternative embodiment of a device according to the present invention that incorporates a photosensitive film as the supporting substrate and isolates the person taking the sample from the sampled material, thereby preventing the transfer of disease.

Figure 9B is a side view of the device of Figure 9A showing the photosensitive film.

Figure 10A is a cross-sectional schematic of an alternative embodiment of a device according to the present invention that uses a coated fiber in which the coating contains immobilized cells.

Figure 10B shows an incubation pouch containing a photosensitive film used for detecting a signal emitted by the cells of the coated fiber of Figure 10A.

Figure 11A is a cross-section of a monofilament coated with immobilized cells.

Figure 11B is a cross-section of a multiple filament thread coated with immobilized cells.

Figure 12 is a schematic of an alternative embodiment of a device according to the present invention that includes a pop-up indicator and a protein or lipid-based glue and immobilized cells containing a protease reporter gene.

Figure 13 is a schematic of preferred template assemblies before cell-coating, after cell coating, and after top-coating. A: Top-view and side-view of template assembly; B: cell-coat patches with surrounding spacer; C: finished top-coated patches.

Figure 14 provides a chart of luciferase activity of latex film-immobilized *E.coli* HB101 containing *mer-lux* constructs after induction by $HgCl_2$ in pyruvate buffer. (A) pRB28. (B) pOS14, (C) pOS15. Symbols: (□) 10,000 nM $HgCl_2$, (○) 1,000 nM $HgCl_2$, (Δ) 100 nM $HgCl_2$, (▽)10 nM $HgCl_2$, (○) 1 nM $HgCl_2$, (+) 0.1 nM $HgCl_2$, and (×) 0 nM $HgCl_2$.

Figure 15 provides a chart of the effect of storage on latex-immobilized *E. coli* HB101 (pRB28). Maximum luciferase activity was plotted as a function of mercury concentration. (A) at -20°C for 3 month. Symbols: (■) immobilized cells freshly made, (▲) immobilized cells stored at -20°C for 3 months in glycerol: PBS buffer (50:50 w/w). (B) at ambient temperature dry 14 days. Symbols: (■) immobilized cells freshly made, (▼) immobilized cells stored ambient temperature for 14 days dry.

Detailed Description of Preferred Embodiments

The present invention provides devices for determining the presence of a metal in a sample. Advantageously, the devices can also quantitatively measure the amount of a metal in a sample. Preferably, the devices are inexpensive and disposable. The metals that can be detected, typically individually detected, and preferably quantitatively measured, include inorganic or organic forms of a variety of metals that can be toxic to humans and other species. These include, for example, mercury (typically, in the form of Hg^{2+} or monomethyl mercury), arsenic (typically in the form of arsenate AsO_4^{3-} or arsenite AsO_2^-), cadmium (typically in the form of Cd^{2+}), antimony (typically in the form of antimonite SbO_2^-), bismuth (typically in the form of Bi^{3+}), and copper (typically in the form of Cu^{2+}).

The devices can be used on liquid samples such as water as well as biological fluids such as blood and urine, for example. Alternatively and significantly, the devices can be used on solid (or semi-solid) samples such as tissue from seafood, particularly fish, clams, crabs, oysters, for example, as well as sludge and soil, for example.

Preferably, and significantly, the devices of the present invention are stable such that the cells remain viable. By stable, it is meant that they are responsive after at least about 8 hours under ambient conditions when the device is in a hydrated condition (during and/or prior to use, the cells are hydrated). More preferably, the devices are stable for at least about 6 months under ambient conditions, and most preferably, indefinitely at a temperature of less than about -10°C, when the device is in a prehydrated condition. The devices of the present invention are also preferably robust such that they can be handled and transported with little or no damage. The

devices can be flexible. Preferably, they include a cell-containing indicator coating that does not delaminate from a supporting substrate, craze, or crack.

The devices of the present invention include immobilized indicating cells. Typically, such cells are immobilized in a polymeric coating and supported on a substrate. This coating with immobilized cells therein is referred to as a "cell-containing indicator coating." Preferably, the devices include a multi-layered construction. A first polymeric layer that includes the immobilized indicating cells is typically supported on a substrate. This layer forms a microporous matrix that entraps whole living cells without adhering to them. The cells can be coated in multiple layers if desired. Optionally, and preferably, the construction includes at least one overlayer of a second polymeric coating. The overlayer prevents the cells from leaving the first polymeric layer upon rehydration of the cells with water or a water-based solution. Other layers are possible as well. Cells immobilized in this manner typically maintain at least about 80% of the original culturability, and preferably have rehydrated culturability that is similar or higher than that of suspended cells when compared over 15 days. Thus, as used herein, a "cell-containing indicator coating" includes one or more cell-containing polymeric layers and one or more polymeric overlayers.

The polymeric layers of the cell-containing indicator coatings may include the same or different polymers. Each polymer used is preferably in the form of a latex, whether it be naturally occurring or synthetic. The polymers can include, for example, acrylate polymers, vinyl acetate polymers, styrene polymers, butadiene polymers, carboxylate polymers, and blends or copolymers thereof. As used herein a copolymer is a polymer of two or more different types of polymers (including copolymers, terpolymers, tetrapolymers, etc.). Such polymers are commercially available from Rohm and Haas of Philadelphia, PA, Dupont of Wilmington, DE, H.B. Fuller Co. of Minneapolis, MN, and GenCorp. of Magadore, OH, for example. Preferably, the polymeric material used for immobilizing the cells includes an acrylic/vinyl acetate copolymer. Preferably, the polymeric material used as an overlayer includes an acrylic/vinyl acetate copolymer.

The polymeric layers (both cell-containing layer(s) and overlayer(s)) can also include additives for various purposes, such as absorbing undesirable material, preventing microbial contamination, and increasing sensitivity. Such additives include, for example, inorganic materials such as $CaCO_3$, aminoacids such as cysteine, pigments such as TiO_2, enzymes such as peroxidase, reactive dyes such as X-gal (5-bromo-4-chloro-3 indolyl-B-D-galactoside),

photoreactive compounds such as silver halides, bacteriostatic compounds such as NaF, antibiotics such as kanamycin or ampenicillin, antimicrobial agents such 1,2-benzisothiazolin-3-one (ICI biocides, Wilmington, DE), osmoprotectants such as sucrose or trehalose or glycerol, biopolymers such as gelatin, carbohydrates such as pyruvate, metals, chemical catalysts, and inorganic mercury absorbent materials. Preferably, the cell-containing layer(s) includes glycerol. Preferably, the overlayer(s) includes glycerol, bacteriostatic compounds, antibiotics, antimicrobial agents, and/or carbohydrates.

Preferably, the cells are bacterial cells, cell clumps or cell mats (i.e., a number of different cells living together is some sort of structure), fungal cells, archea cells, or eukaryotic cells. Examples of suitable cells include bacterial cells such as *E. coli* and *Streptomyces anulatus*, *Thermotoga*, archea cells such as *Pyrococcus*, eukaryotic cells such as bakers yeast and *Penicillium chrysogenum*, as well as plant cells.

The cells are preferably genetically engineered to produce a response, such as a mechanical or chemical response, and thereby emit a signal that can be detected. Examples of suitable responses include, but are not limited to, luminescence, production of an enzyme or metabolite, evolution of heat, or a change in H^+, OH^-, or reactive radical concentration. This response occurs upon contact with the metal being detected. Significantly, many of these responses can be measured such that the metal can be quantitatively measured.

Preferably, the cells are recombinant *E. coli, Bacillus,* or *Streptomyces* cells that include a metal resistant promoter, such as a mercury resistant promoter, and a reporter gene encoding a protein such as, for example, luciferase, protease, lipase, gluco-isomerase or green fluorescent protein. Preferably, the cells include a bioluminescent operator/promoter *mer-lux* plasmid, although other operator/promoter constructs can be used including *ars-lux, smt-lux,* and *cad-lux.*

Several *mer-lux* plasmid constructs are known. The constructs made by Selifonova et al. (Appl. Environ. Microbiol., 59, 3083-3090, (1993)) are particularly useful in that each construct has been tested extensively for mercury sensitivity in suspended cultures under different conditions. These plasmids are pRB28, pOS14, and pOS15. They all code for luciferase activity (luxCDBE) but differ in the subset of *mer* genes fused to the lux gene. pRB28 contains *merR* (the *mer* repressor gene) and a truncated *merT (*one of the mer transporter genes). A second construct, pOS14, contains merR and the complete set of mercury transport genes *merT, merP, and merC.* The third construct, pOS15, contains

merRTPC, the reductase gene *(merA)* and a second regulatory gene (merD). Induction of the *mer* operon by inorganic mercury results in the production of luciferase which can be assayed by the ATP-dependent emission of photons.

Preferably, the polymeric layers are supported on a substrate that enables detection and/or measurement of the metal by monitoring the signal produced upon the cells responding to contact with the metal (e.g., luminescence). For such preferred embodiments, the supporting substrate can be a photosensitive film or a light-sensitive electronic chip, for example. Alternatively, the substrate can merely support or protect the cells and not take part in the detection of the metal. For such embodiments, the supporting substrate can be a solid light impenetrable backing, for example. In such embodiments in which the supporting substrate does not detect the signal emitted by the cells, the cells would need to be brought into close proximity to a detector. Such a detector could be, for example, a photosensitive film that does not have a cell-containing indicator coating thereon, a scintillation counter, or light meter. This occurs in the embodiments described below with respect to Figures 3, 8, and 10.

The methods of detection include various detection mechanisms. For example, such methods can involve detecting light fluorescence as from expression of Lux, a fluorescent protein, detecting the hydrolytic enzyme activity such as protease activity, detecting the production of a metabolite, detecting the evolution of heat, detecting a change in H^+, OH^-, or reactive radical concentration change, detecting the evolution of a gas such as carbon dioxide, detecting the utilization or depletion of a substrate such as glucose, and/or detecting a change of color. A preferred method is the expression of Lux.

The polymeric materials, with or without cells incorporated therein, may be coated on the substrate by a wide variety of methods, including, for example, draw down coating, slot coating, die coating, spin coating, gravure coating, or ink jet or laser jet printer coating. Typically, the cell-containing layer(s) are dried prior to the overlayer(s) being applied. The coating process can be carried out at temperatures varying from about 4°C to about 95°C. The coating method preferably provides good control over cell density, and coating thickness which leads to easily standardized responses or measurements. Alternatively, however, the layers can be simultaneously coated or coated sequentially without intervening drying steps, if so desired.

The devices may optionally include a removable film (a "top" film) that protects the cell-indicating coating. This top film is typically a layer of foil, although it could be a layer of cellulose acetate, or a wide variety of other synthetic or natural materials. The devices may also optionally include a removable film (a "bottom" film) that protects the supporting substrate, such as a light-sensitive electronic chip.

Various embodiments of the devices of the present invention are described by reference to the figures. Each of these devices is described with respect to the detection of mercury (whether it be in the form of inorganic mercury such as Hg^{2+} or organic mercury such as monomethyl mercury), although other metals could be detected. Also, many of these devices are described with respect to the detection of mercury in fish tissue or fluids, although other samples could be tested. Furthermore, except for the device of Figure 12, each of the embodiments includes cells that emit light upon exposure to mercury. Again, this is only for illustration purposes as other types of cells can be used and the devices modified accordingly, which would be readily apparent to one of skill in the art upon reading the teachings herein.

Figure 1A shows a basic structure for a device according to the present invention. The device 10 includes a cell-containing indicator coating 12 (having immobilized cells therein) coated on a photosensitive film 14 (the supporting substrate). The device also includes removable protective films 16 (first or top film) and 18 (second or bottom film), which may be made of foil and may or may not include pull tabs (which is shown for film 16). Figure 1B is a bottom view of the photosensitive film 14 (such as a commercially available sheet of POLAROID film) after the film 18 is removed showing a photosensitive area 17, which displays a response to mercury. Also shown is a built-in photodensity measuring device 19, which is used as a comparator. For example, the photodensity measuring device 19 can be a strip of material, such as paper or plastic, having printed thereon an image with increasing black density graduated to correspond to the level of mercury detected. The photodensity measuring device 19 also could be a mercury-containing compound spread on the surface at an increasing concentration. In this way, the device would include an internal standard for quantifying the level of mercury. Thus, in both cases the strip would be labeled with the mercury concentration. In use, the top film 16 is removed to expose the immobilized cells to the sample of interest. The immobilized cells, sample of interest, and nutrients (such as glucose in the presence of a buffer, for example) are incubated

(for example, under ambient conditions for at least about 15 minutes). The bottom film 18 is then removed and the level of mercury (which may be in the form or inorganic mercury and/or monomethyl mercury) is determined by viewing the photosensitive area 17 and comparing it to the photodensity measuring device 19.

Figure 2A shows an alternative construction for a device 20 in which a cell-containing indicator coating 22 (having immobilized cells therein) is coated on a light-sensitive electronic chip 24. The device also includes removable protective film 26, which may or may not include a pull tab (which is shown for film 26). Figure 2B is a bottom view of the electronic chip 24, which may be reusable, and has an electrical connection 25. In use, the film 26 is removed to expose the immobilized cells to the sample of interest. The immobilized cells, sample of interest, and nutrients are incubated as described above. The level of mercury is measured with a voltmeter (not shown). The light generated by the cells is measured by the voltage generated by the light sensitive chip.

Figure 3 shows an alternative structure for a device 30 in which a cell-containing indicator coating 32 (having immobilized cells therein) is coated on a backing 34 that does not detect the signal (e.g., light, heat, etc.) produced by the cells. The backing shown is not light transmissive, although this is not a necessary requirement. The device also includes removable protective film 36, which may or may not include a pull tab (which is shown for film 36). In use, the film 36 is removed to expose the immobilized cells to the sample of interest. The immobilized cells, sample of interest, and nutrients are incubated as described above. The light generated by the cells is measured with an external meter, such as a scintillation counter or light meter. The mercury is correlated to the light level. Hand-held or laboratory meters are available.

Figure 4 shows a device 40 that can penetrate into a sample of interest, such as fish tissue, through the scales, skin, and into the tissue. The penetration device 40 includes the construction shown in Figure 1, which includes a cell-containing indicator coating 42 coated on a photosensitive film 44 (or alternatively, it could include the constructions shown in Figures 2 or 3). The device also includes removable bottom film 48 (and a top film, which is not shown). In use, a cut is made in the fish, the top film (not shown) that protects the cell-containing indicator coating 42 is removed, device 40 is inserted into the cut and placed there for a predetermined length of time to sorb the mercury. The device is removed and incubated

with nutrients as described above. The level of mercury is then measured by evaluating the photosensitive film 44.

Figure 5 shows yet another construction for a device 50 according to the present invention. The device 50 is in the form of a container, such as a pouch. It includes a cell-containing indicator coating 52 (having immobilized cells therein) coated on a supporting substrate 54, such as a photosensitive film or a light-sensitive electronic chip, for example. The device also includes removable protective films 56 and 58. The top film 56 is not in direct contact with the cell-containing indicator coating 52; rather, it is spaced above it, thereby forming a cavity 59 for placement of a sample 57, such as a slice of fish tissue. In use, the film 56 is removed and the sample 57 is placed in the cavity 59 in direct contact with the cell-containing indicator coating 52. The immobilized cells, sample, and nutrients are incubated as described above. The film 58 is then removed and the level of mercury is measured using the support 54.

Figure 6 shows a very similar device to that shown in Figure 5. In this embodiment, the sample of interest, such as fish tissue need not be placed in the cavity; rather an absorbent pad 65, which can be made of cellulose or sponge, for example, containing fluids from the fish tissue can be used. The device 60 includes a cell-containing indicator coating 62 coated on support 64, which can be a photosensitive film or a light-sensitive electronic chip, for example. The device also includes removable protective films 66 and 68. As in the device shown in Figure 5, the top film 66 is not in direct contact with the cell-containing indicator coating 62; rather, it is spaced above it, thereby forming a cavity 69 for placement of an absorbent pad 65. In use, the film 66 is removed and the absorbent pad 65, which had been in contact with a slice of fish 67 to absorb fluids from the fish tissue, is placed in the cavity 69 in direct contact with the cell-containing indicator coating 62. The immobilized cells, absorbent pad, and nutrients are incubated as described above. The film 68 is then removed and the level of mercury is measured using the support 64.

Figure 7A shows a device 70 that includes the construction shown in Figure 1 (or alternatively, it could include the constructions shown in Figures 2 or 3), that includes a coring device 71 that is inserted into a container 73. The container 73 includes a reservoir 75, which is filled with nutrients and buffer. The bottom of the container 73 (or optionally the sides of the container) includes a cell-containing indicator coating 72 coated on a photosensitive film 74. As shown in Figure 7B, the

coring device 71 is designed to penetrate fish scales, skin, and tissue, as described above for Figure 4. It is used to remove a core sample of fish tissue and deliver this core sample to the reservoir 75 in container 73. Both the coring device 71 and the container 73 can have threads such that the coring device 71 is screwed into the container, although this is not a requirement. The mercury from the tissue sample migrates to the immobilized mercury sensitive cells located in the cell-containing indicator coating 72 in the bottom (or sides) of the container 73. The reservoir 75 includes nutrients in a buffer for incubation of the cells. The light generated by the cells is detected by a photosensitive film 74 (e.g., POLAROID film) attached to the bottom (or sides) of the nutrient reservoir 75.

Figure 8A shows a device similar to that shown in Figure 7, except that the coring device 81 includes a cell-containing indicator coating 82 (having immobilized cells therein) directly on the coring device 81. Also, the coring device 81 does not need to be hollow. Thus, it does not necessarily remove a core tissue sample from a fish as described with respect to Figure 7. In this embodiment and as shown in Figure 8B, the immobilized cells located in the tip of the coring device 81 are exposed to the fish tissue while it is inserted in the fish tissue. After exposure to the fish tissue, the coring device 81 is inserted into the reservoir 85 of container 83 where the cells are incubated in buffer and nutrients. The light generated by the cells is detected by a photosensitive film 84 (e.g., POLAROID film) attached to the bottom (could also be the sides) of the nutrient reservoir 85.

Figure 9 shows a device that allows for a sample to be drawn into a reservoir, such as into a syringe. In device 90 there is a construction as described above for Figure 1. In Figure 9A, a side view shows a cross-section of the device 90 that includes a cell-containing indicator coating 92 coated on a photosensitive film 94. The device also includes removable protective film 98. The device 90 also includes a syringe needle 91, a reservoir 93, and a bulb 95, which can be used to draw up a liquid sample into the reservoir 93. The cell-containing indicator coating 92 is positioned inside the reservoir with photosensitive film 94 and protective film 98 forming an external wall. Figure 9B is a bottom view of the photosensitive film 94 (e.g., POLAROID film) after the film 98 is removed showing a photosensitive area 97, which displays a response to mercury. Also shown is a built-in photodensity measuring device 99 as described above with respect to Figure 1. In use, a

liquid sample is drawn into the reservoir 93, where it comes in contact with the immobilized cells of the cell-containing indicator coating 92. Nutrients are added and the cells and sample are incubated. The film 98 is then removed and the level of mercury is measured by evaluating the photosensitive area 97 as it compares to the photodensity measuring device 99.

Figure 10 discloses a device that includes a cell-containing indicator coating coated on a fiber 100, composed of, for example, polyester, nylon, cellulose acetate, or an optical fiber. As shown in Figure 10A, the coated fiber 100 has an end-stop 101 and a penetrating end 103. The device is shown penetrating through a fish tail. Once a sufficient amount of time has lapsed for the immobilized cells of the cell-containing indicator to be in contact with the fish tissue, the coated fiber 100 is removed. As shown in Figure 10B, the coated fiber 100 is then placed in contact with a photosensitive film, which can be in a pouch or container, for example, having nutrients and buffer therein. After a sufficient incubation period, the mercury can be qualitatively detected by viewing a photographic image 105 of the thread on the photosensitive film.

Figure 11A shows a cross-section of a coated fiber 100, as shown in Figure 10. The coated fiber 100 includes a monofilament 106 coated with a cell-containing indicator coating 102. The cell-containing indicator coating 102 includes a cell-containing polymeric layer 107 and a polymeric overlayer 108. Figure 11B shows a cross-section of a coated fiber 110 that includes a multiple filament thread 116 with a cell-containing indicator coating 112. The cell-containing indicator coating 112 includes a cell-containing polymeric layer 117 and a polymeric overlayer 118. The coated fiber 110 may also include a polymeric precoat layer 119 between the multiple filament thread 116 and the cell-containing indicator coating 112.

Figure 12 shows a device 120 in which cells are used that contain a plasmid with a mercury resistance promoter that activates a gene to produce a secreted protease or lipase. The cells are included in a cell-containing indicator coating 122. A pop-up indicator rod 121 is initially held in place by a protein or lipid based glue 123. The pop-up indicator rod 121 may be used either qualitatively or quantitatively, if it includes an indicator scale (not shown). The device also includes a spring 125 under tension that is attached to the pop-up indicator rod 121. In use, the device 120 is inserted into fish tissue. When mercury is detected by the cells in the cell-containing indicator coating 122 as it diffuses through a perforated housing 126 of device 120, the resulting protease or lipase degrades the glue 122 and

releases the rod 121. As a result of the tension placed on the rod 121 by the spring 125, the rod moves up. By the design of the rod, the glue, and the glue holder the device could be made to be quantitative. Alternately, the pop-up indicator rod 121 could be attached to a piston to measure the production of a gas. In that case the cells would contain the genes needed to produce high levels of gas when activated. In an alternative embodiment, the cell-containing indicator coating could include the protein or lipid based glue in place of, or in addition to, the polymer used to make the coating.

Examples

Method for Preparing Mercury Biosensors Films of *Escherichia coli*

Bacterial Strains and Media. The methods procedures and techniques are substantially those found in well known molecular cloning and genetics guides such as Maniatis et al., Molecular Cloning; a Laboratory Manual, Cold Spring Habor, N. Y., 1982. Materials are commercially available from sources such as GIBCO/BRL, Gathersburg, Md, or Promega, Madison, WI. *E. coli* HB101 containing pRB28, pOS14, or pOS15 are in the public but could be constructed through these cloning methods as described by Selifonova et al., Appl. Environ. Microbiol., 59, 3083-3090, (1993)).

Coating Materials. Harvested *Escherichia coli* HB101 cells were mixed with glycerol and acrylic/vinyl acetate copolymer latex (Rohm and Haas, Philadelphia, PA) in a ratio depending on the number of cells immobilized. Commonly used was 1.2 g cell paste: 0.3 ml 50% (w/w) glycerol: 1 ml latex, which were mixed together. The cell-polymer mixture was coated onto a polyester template (e.g., pressure sensitive adhesive tape) using a 26 mil wire wound rod (Mayer bar) at 4°C, as described in greater detail below. After the coated layer was dry, the template was removed, and a second layer of latex (overlayer) was coated on top. The topcoat layer was dried at 4°C, and the assembly was cured at 37°C for 30 minutes or shorter. Individual patches were excised and rehydrated in buffer (5 mM pyruvate, NaK-phosphate buffer pH 6.8 [34 mM sodium phosphate; 33 mM

potassium phosphate, pH adjusted to 6.8] and 0.091 mM $(NH_4)_2SO_4$). The preparation of the template and coating procedure is described in detail below and with reference to Figure 13.

Coating Method. A punch was used to create circular holes in a pressure sensitive tape such as that obtained from Minnesota Mining & Manufacturing Company, St. Paul, MN, under the trade designation SM 7830 to form a template. The template was rolled onto a substrate of similar length and width. This creates uniform circular wells of a desired diameter and depth (12.7 mm diameter and 42.6 μm well depth was commonly used), as shown in Figure 13A. A pool of cell-polymer mix was delivered at the top of the template in a line spanning the width of the patch area. A 26 mil wire diameter Mayer rod was placed above and then drawn through the coating liquid and across the wells formed by the template. The cell-coat was dried until no moisture was visible. At 4°C and 70% relative humidity, drying took 2 hours. The template was then peeled off. As shown in Figure 13B, a spacer consisting of a pressure sensitive tape was placed around the newly formed cell-coat patches to prevent the Mayer rod from touching during coating. Commonly used spacer thickness was 155 μm. The spacer was laid down on all four sides of the substrate containing the cell patches to contain the top-coating liquid during drying. A pool of sealant liquid (latex with 5% glycerol) was delivered in line spanning the width of the area enclosed by the spacer and coated with a 26 mil wire diameter Mayer rod. After the top-coat had dried, the spacer was removed, leaving the construction shown in Figure 13C. The top-coat was dried at 4°C and 70% relative humidity for 2.5 hours until it took on a transparent and matte appearance.

Detection of Mercury.

Figure 14

Section A. Patches of immobilized *E.coli* HB101 harboring the pRB28 *mer-lux* constructs were analyzed individually in 20 ml scintillation vials in a liquid scintillation counter, (single photon counting mode, 1 minute counting time, Beckman, LS 7000, Columbia, MD) for luciferase activity after exposure (the patches were submerged in the liquid containing the mercury) to $HgCl_2$

concentrations from 0.1 nM to 10,000 nM. Luciferase activity induced by 0.1, 1, or 10 nM $HgCl_2$ was not apparent during the first 5 hours of induction but increased substantially during the next 15 hours after which time the activity continued to increase or remained constant until 37 hours. *E. coli* HB101 (pRB28) exposed to higher levels of Hg(II) had significantly different kinetics of luciferase induction. At 100, 1,000 or 10,000 nM $HgCl_2$, the luciferase activity reached maximum detection levels (limited by the scintillation counter to 6×10^6 count of single photons per minute) within the first 5 hours of induction.

Section B. Patches of immobilized *E.coli* HB101 harboring the pOS14 *mer-lux* constructs were analyzed individually for luciferase activity after exposure to $HgCl_2$ concentrations from 0.1 nM to 10,000 nM, At 0.1 nM $HgCl_2$. Light induction was not significant compared to the control. Hg(II) concentrations at 1 nM and 10 nM induced luciferase activity after a 4-5 hour lag, and luciferase activity increased during the next 10 hours of the assay. At 100, 1,000 and 10,000 nM $HgCl_2$, luciferase activity was evident after 2 hours of incubation and reached the maximum detectable (6×10^6 count of single photons per minute) after 5-8 hours.

Section C. Patches of immobilized *E.coli* HB101 harboring the pOS15 *mer-lux* constructs were analyzed individually for luciferase activity after exposure to $HgCl_2$ concentrations from 0.1 nM to 1,000 nM. The only concentration of Hg(II) that showed significant luciferase induction above background levels with pOS15 was 1,000 nM $HgCl_2$.

Figure 15

Patches containing *E. coli* HB101 (pRB28) cells that were identical to those used for $HgCl_2$ induction of luciferase activity in pyruvate buffer were stored at -20 °C for 3 months or as dry (meaning nonrehydrated) for 14 days. Samples were then either thawed or rehydrated in pyruvate phosphate buffer mentioned above and exposed to $HgCl_2$. The induced maximum luciferase activity was compared to freshly prepared immobilized cell patches. Freezing the patches did not significantly affect luciferase induction significantly. Maximum sensitivity for frozen stored patches was 0.1 nM $HgCl_2$ equal to that of non-stored patches. Also the range (0.1 to

100 nM $HgCl_2$) in which a detectable change in signal was observed was unchanged. Storage 14 days dry decreased the maximum luciferase activity observed between 100 nM and 0.1 nM $HgCl_2$. The maximum sensitivity decreased from 0.1 nM to 1 nM Hg Cl_2. The range in which there was a detectable change in signal was expanded to the range 1 nM to 10,000 nM $HgCl_2$.

The complete disclosures of the patents, patent documents, and publications cited herein are incorporated by reference in their entirety as if each were individually incorporated. Various modifications and alterations to this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention. It should be understood that this invention is not intended to be unduly limited by the illustrative embodiments and examples set forth herein and that such examples and embodiments are presented by way of example only with the scope of the invention intended to be limited only by the claims set forth herein as follows.

WHAT IS CLAIMED IS:

1. A device for determining the presence of a metal in a sample comprising a cell-containing indicator coating supported on a substrate, wherein, upon contact with the metal, the cells produce a response and emit a signal.

2. The device of claim 1 wherein the cell-containing indicator coating comprises cells immobilized in one or more layers of a polymeric material.

3. The device of claim 2 wherein the cells are bacterial cells.

4. The device of claim 3 wherein the bacterial cells are *E. coli* cells.

5. The device of claim 2 wherein the cells are genetically engineered to produce a response to the metal of interest.

6. The device of claim 5 wherein the cells luminesce upon contact with the metal of interest.

7. The device of claim 6 wherein the cells include a metal resistant promoter and a reporter gene that encodes luciferase.

8. The device of claim 1 which is capable of detecting a metal in an inorganic or organic form.

9. The device of claim 8 which is capable of detecting mercury.

10. The device of claim 9 which is capable of detecting Hg^{2+} or monomethyl mercury.

11. The device of claim 1 wherein the substrate is capable of detecting the signal.

12. The device of claim 11 wherein the substrate is a photosensitive film or a light-sensitive electronic chip.

13. The device of claim 1 wherein the substrate supports the cells but does not detect the signal.

14. The device of claim 13 wherein the substrate is a fiber.

15. A device for determining the presence of mercury in a sample comprising a cell-containing indicator coating supported on a substrate, wherein, upon contact with the mercury, the cells produce a response and emit a signal that is detected by the substrate.

16. The device of claim 15 wherein the substrate is a photosensitive film or a light-sensitive electronic chip.

17. The device of claim 15 which is incorporated into a housing that is capable of penetrating a solid sample.

18. The device of claim 17 wherein the sample is fish tissue.

19. The device of claim 15 which is capable of quantitatively measuring the amount of mercury in a sample.

20. A method of determining the presence of a metal in a sample, the method comprises contacting the sample with a device comprising a cell-containing indicator coating supported on a substrate, wherein, upon contact with the metal, the cells produce a response and emit a signal; and detecting the signal.

DEVICES FOR MEASURING METALS

Abstract of the Disclosure

The present invention provides devices for measuring metals, particularly toxic metals such as mercury. The devices include a cell-containing indicator coating supported on a substrate, wherein, upon contact with the metal, the cells produce a response and emit a signal.

Fig. 1

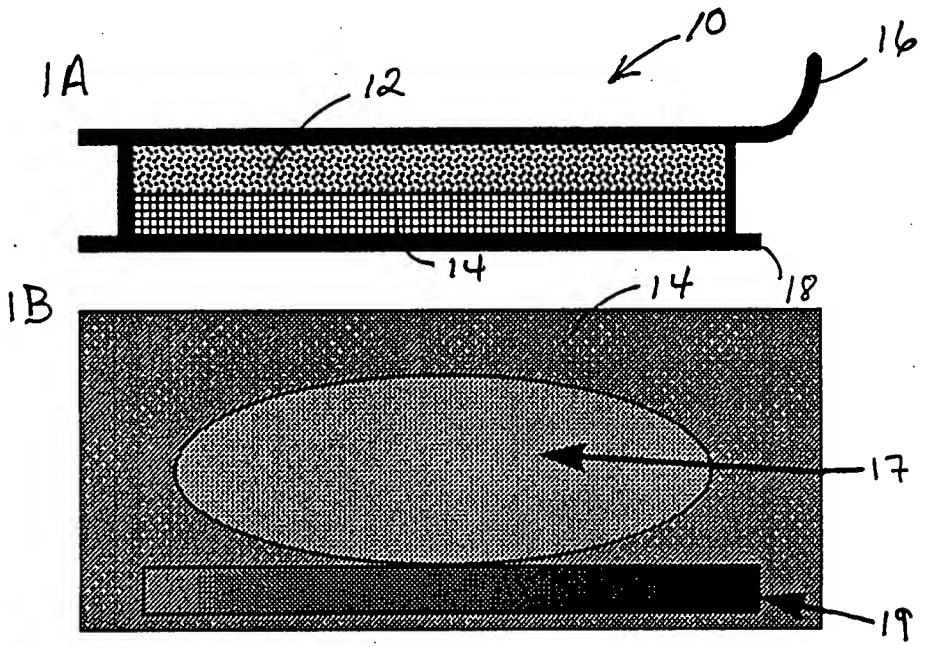


Fig 2

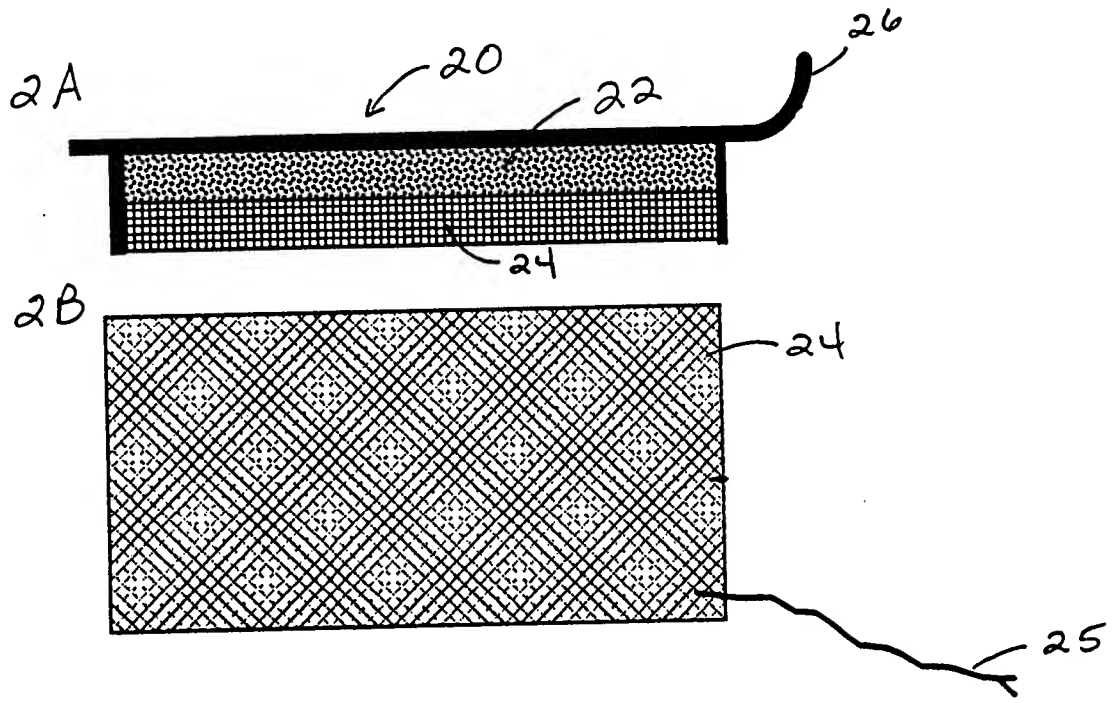


Fig. 3

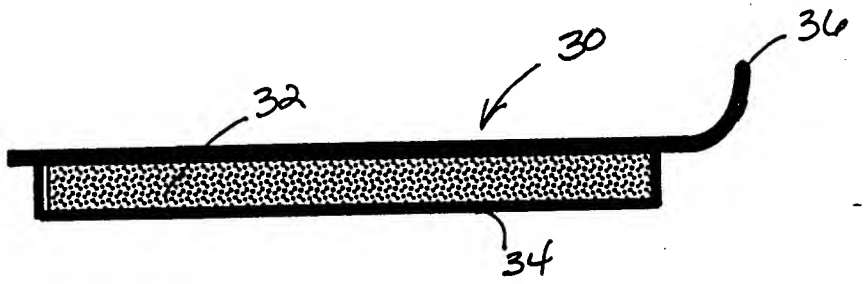


Fig. 4

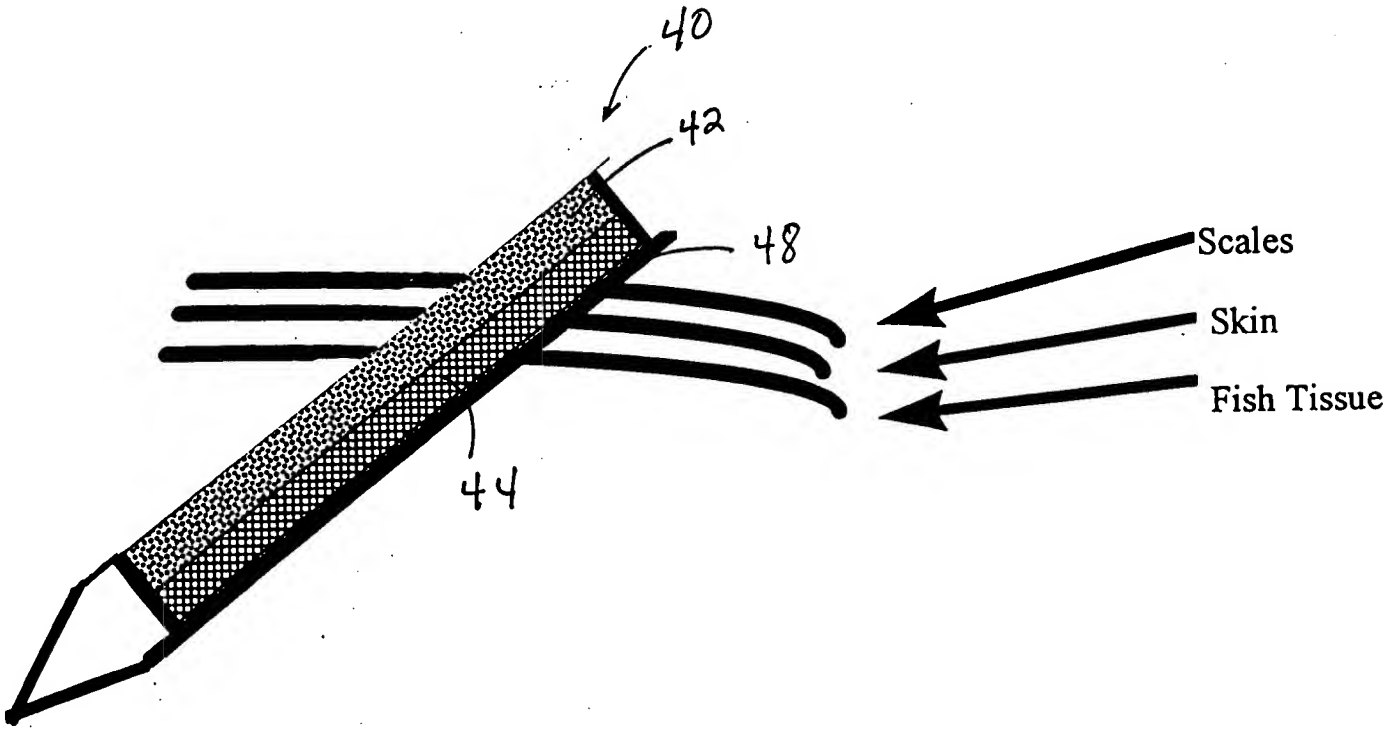


Fig. 5

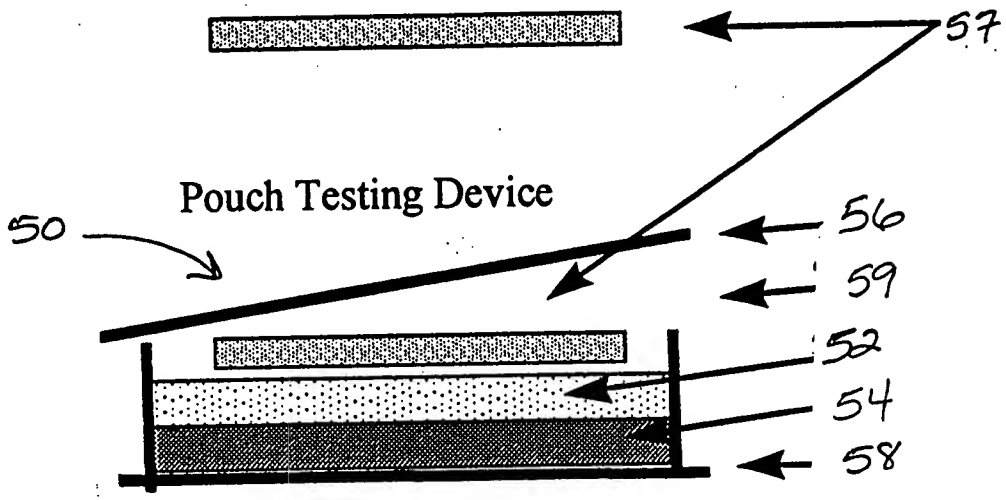


Fig. 6

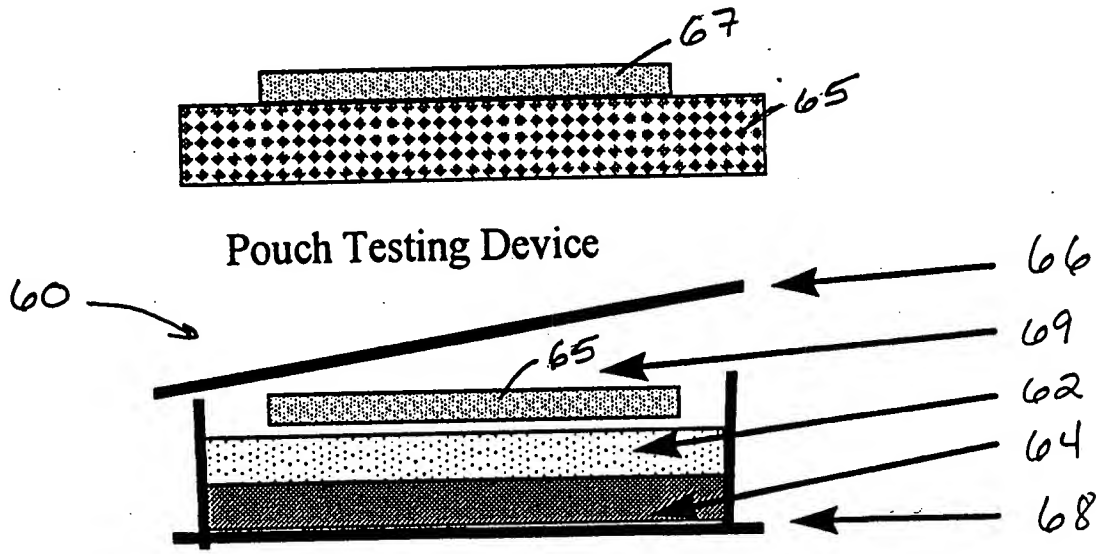


Fig. 7

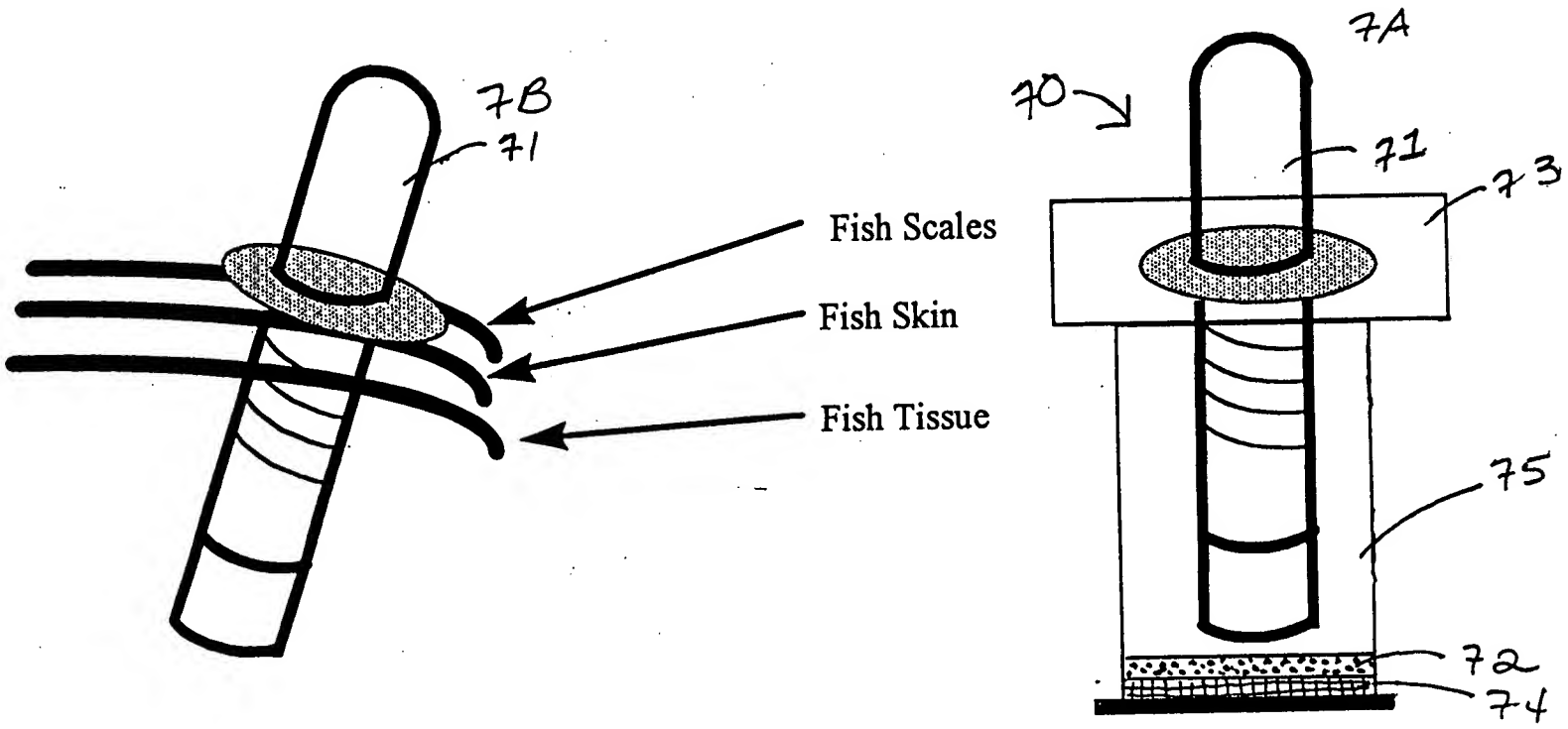


Fig. 8

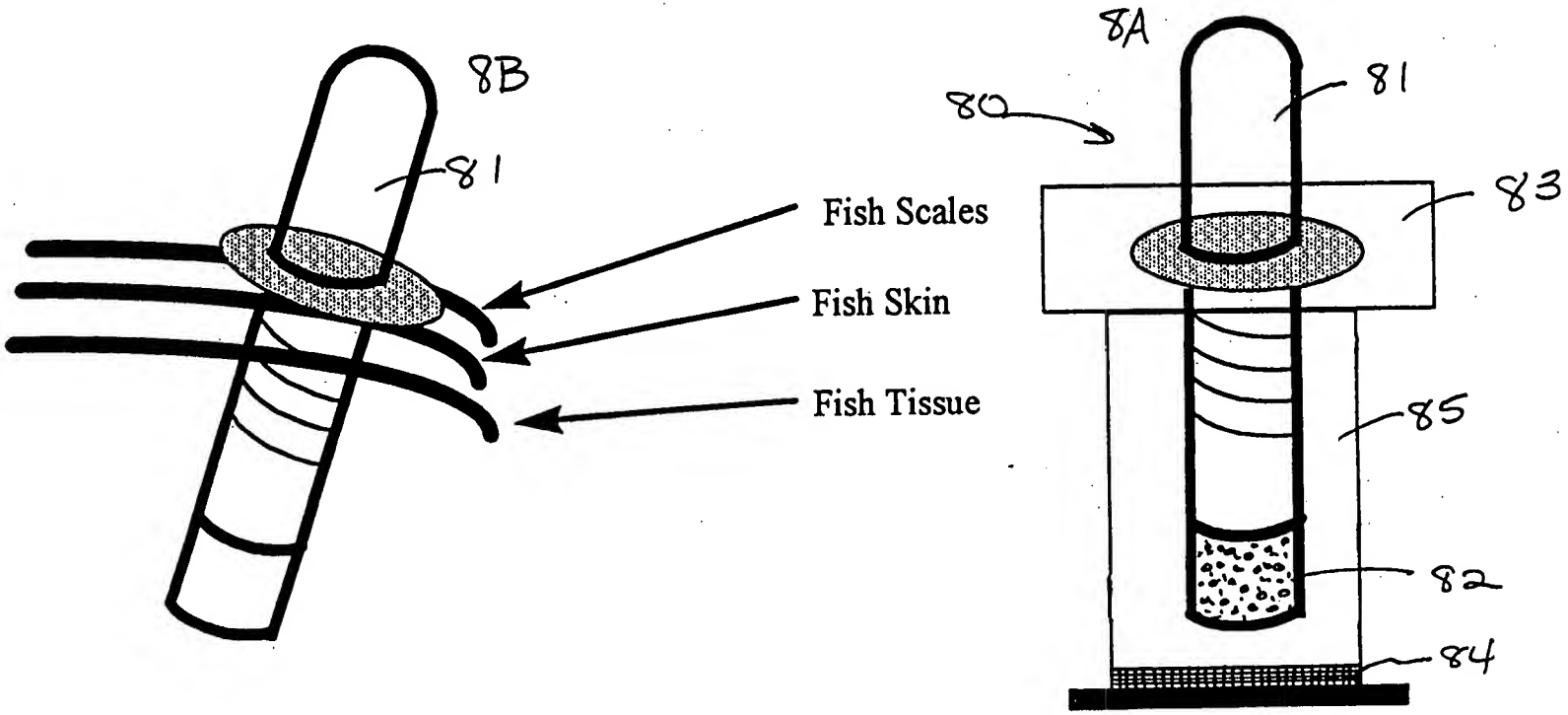


Fig. 9

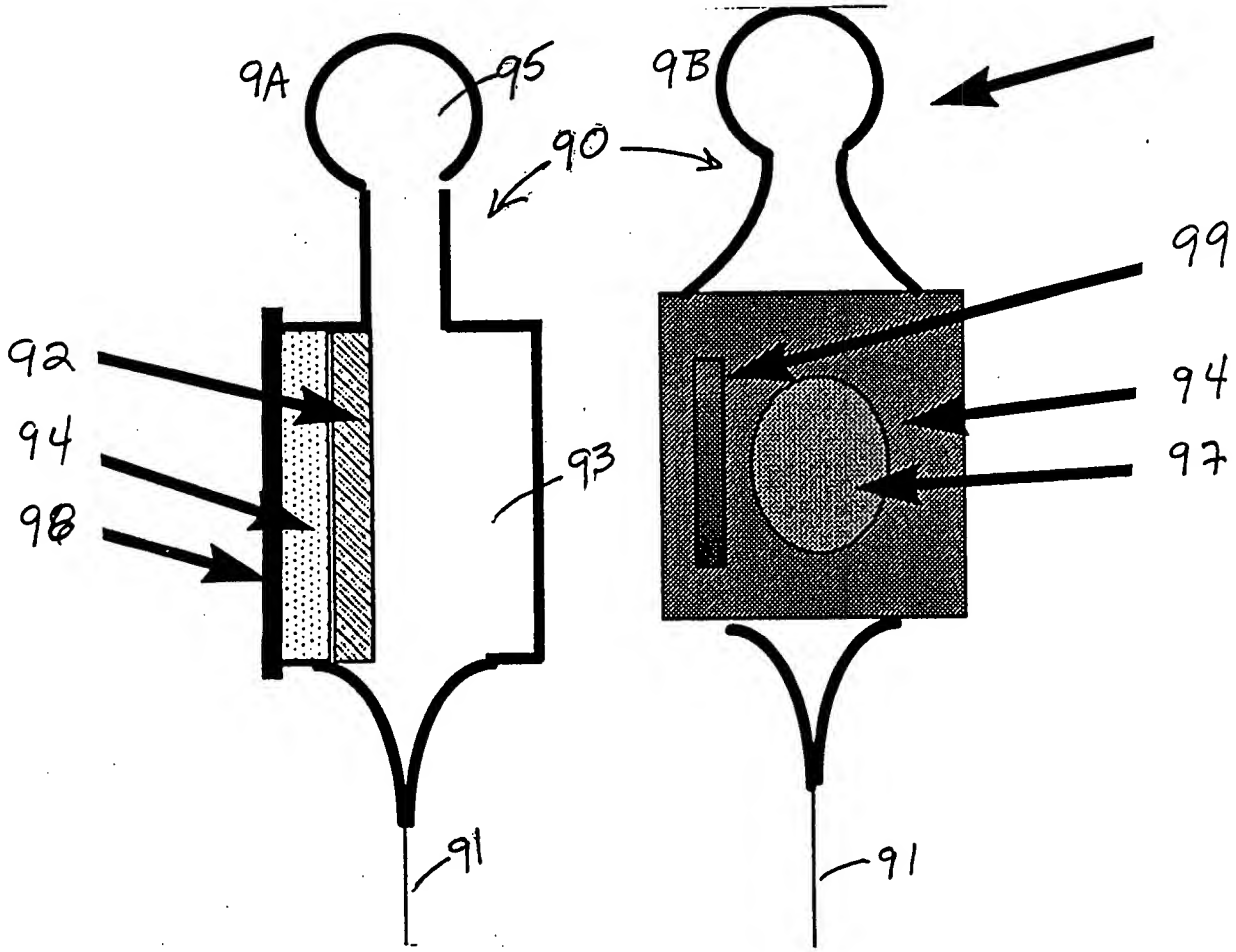


Fig. 10

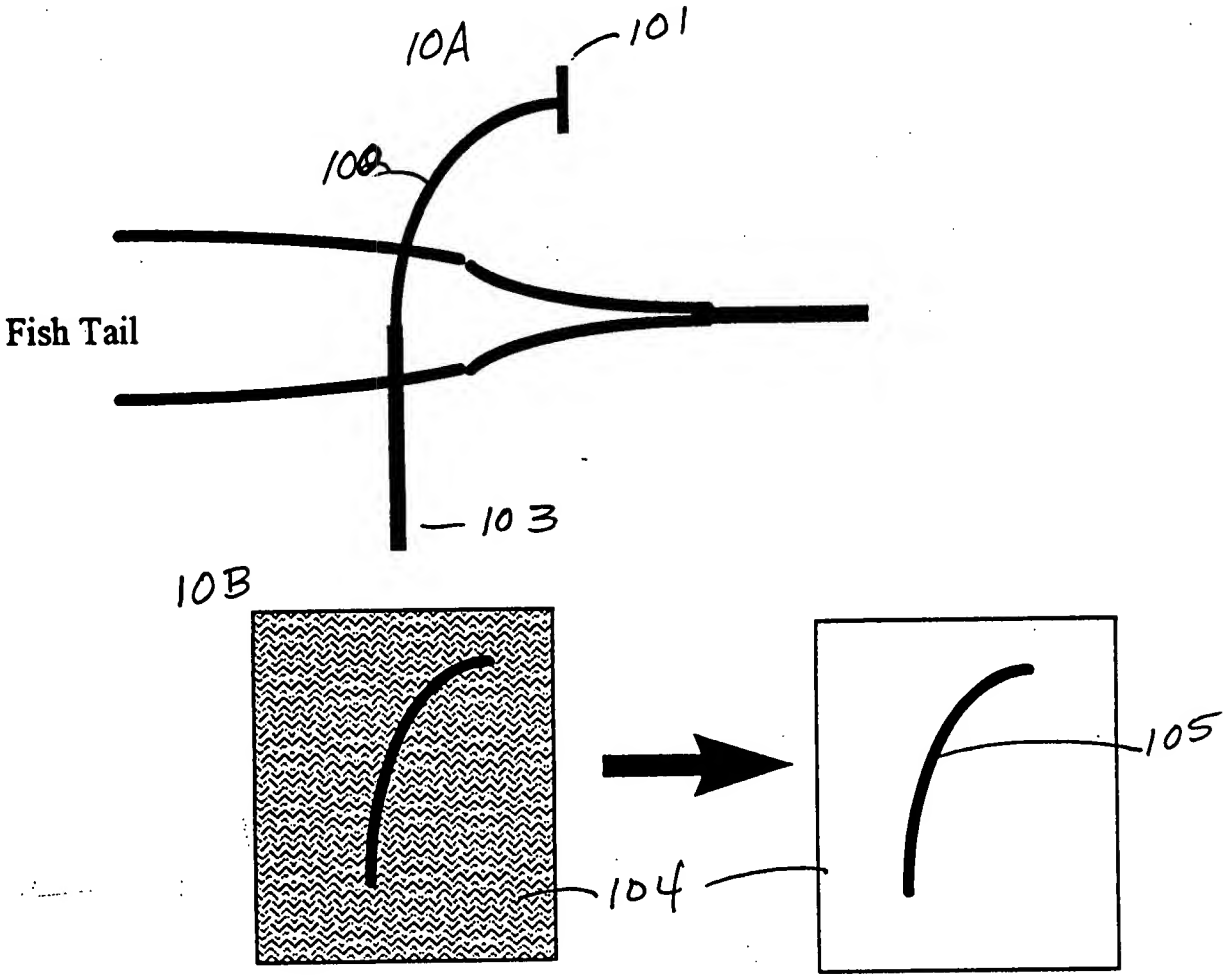
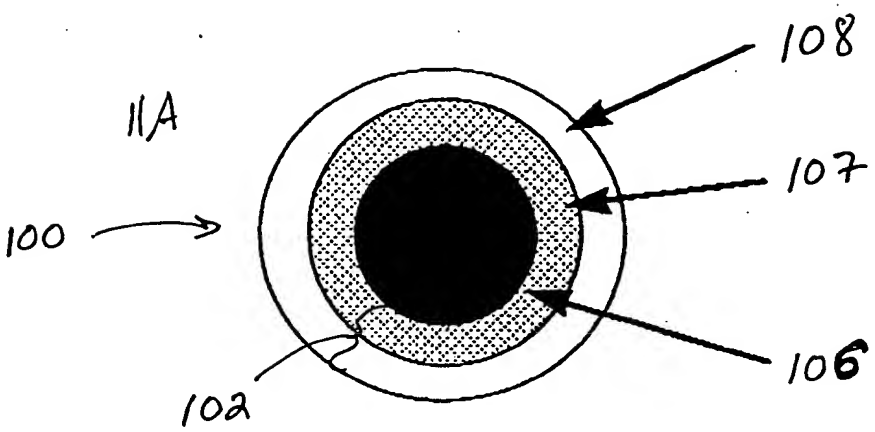
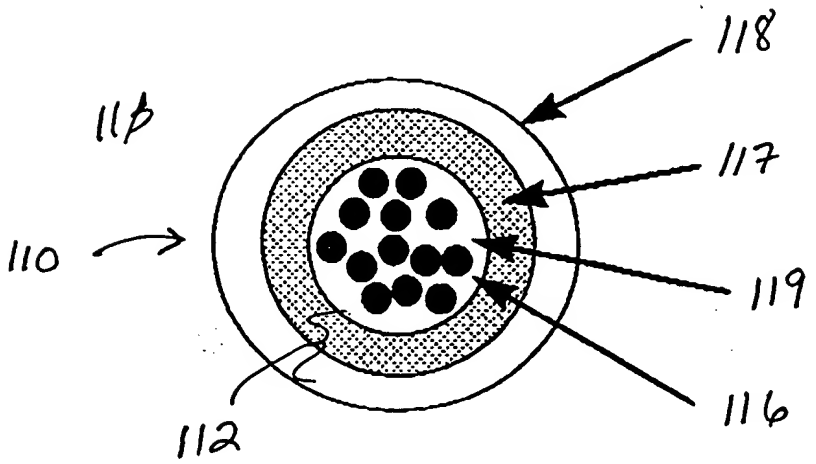


Fig. 11



Coating on a Mono-filament



Coating on a Thread
Containing Multiple Filaments

Fig. 1a

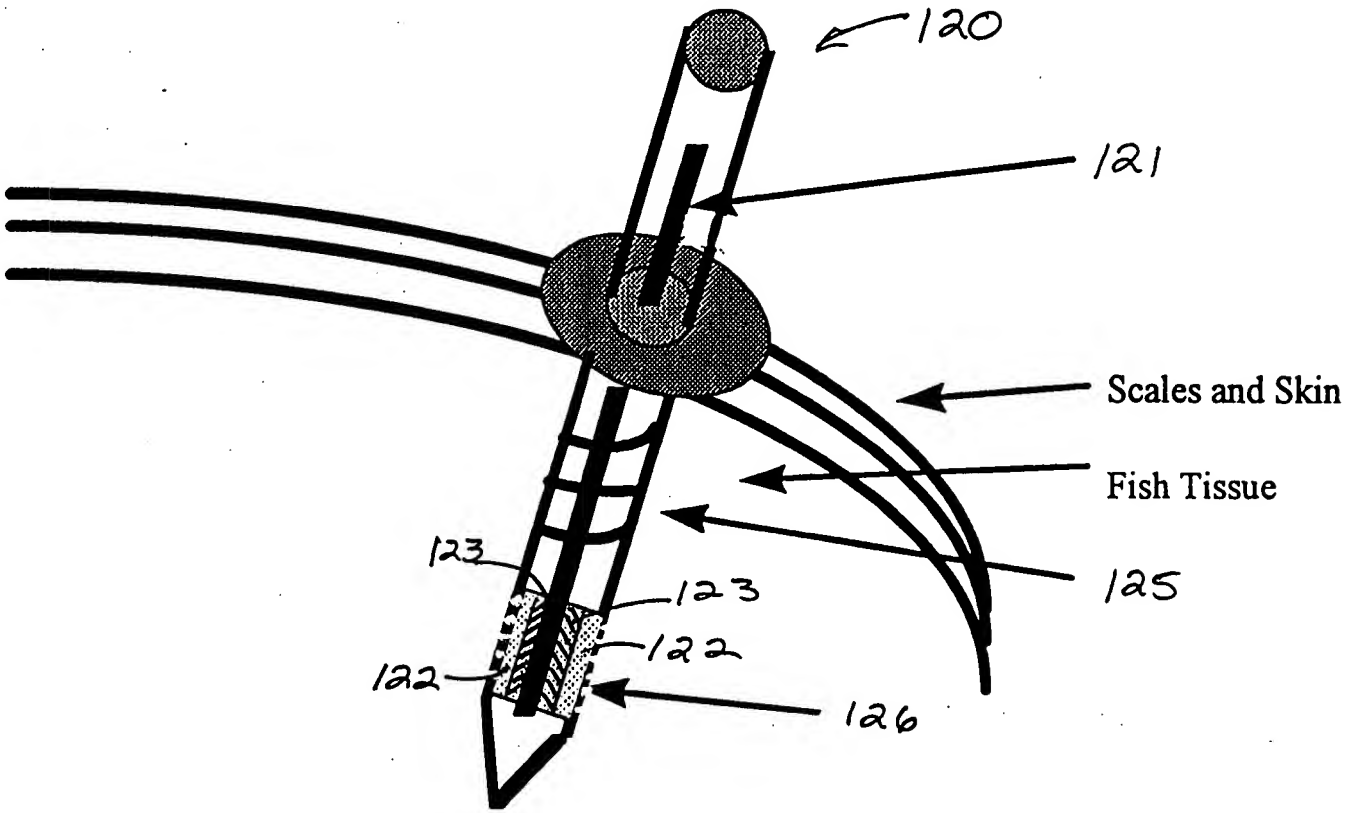


Fig. 13

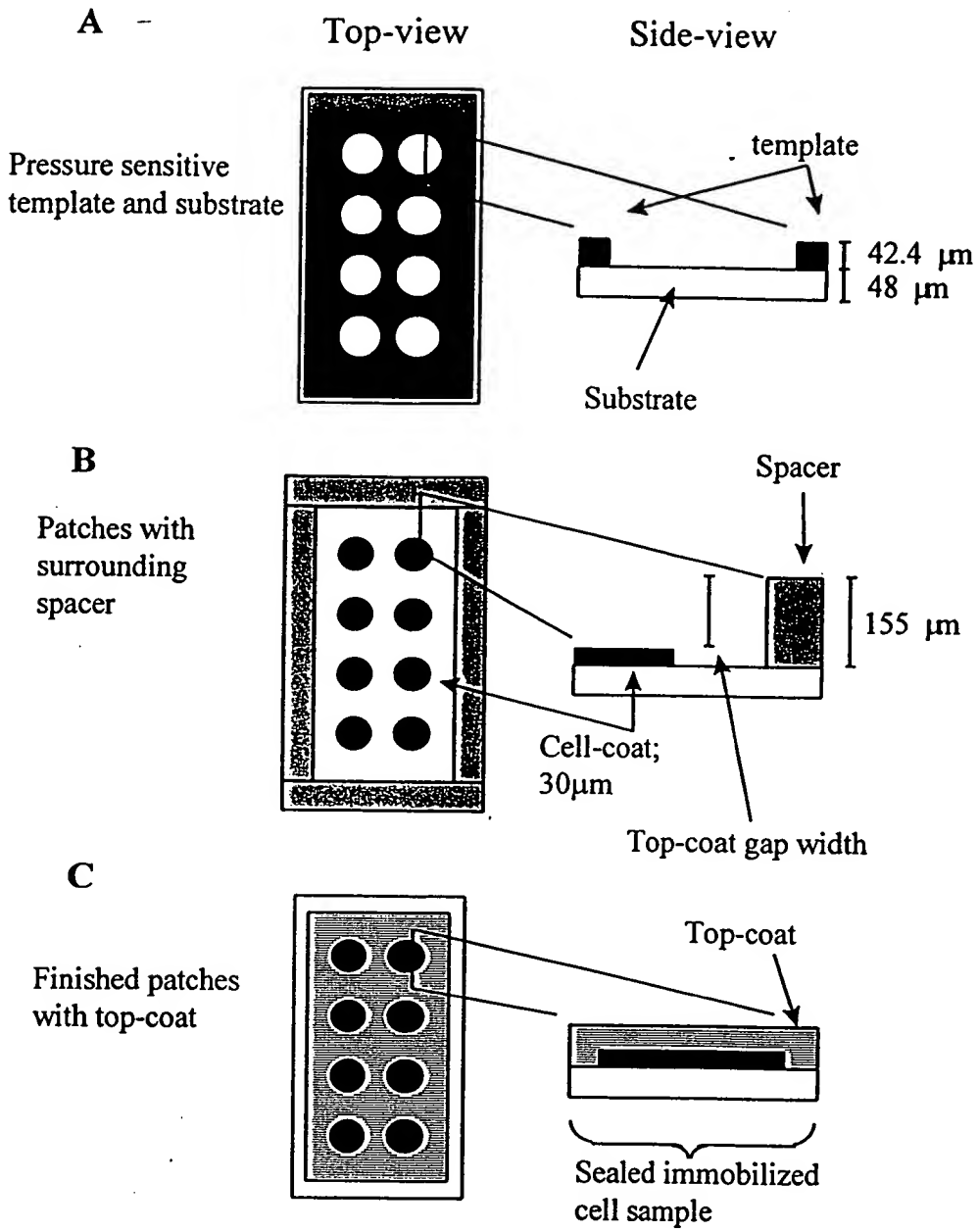

A
Top-view
Side-view
Pressure sensitive template and substrate
template
42.4 µm
48 µm
Substrate
B
Patches with surrounding spacer
Spacer
155 µm
Cell-coat; 30µm
Top-coat gap width
C
Finished patches with top-coat
Top-coat
Sealed immobilized cell sample

Fig. 14

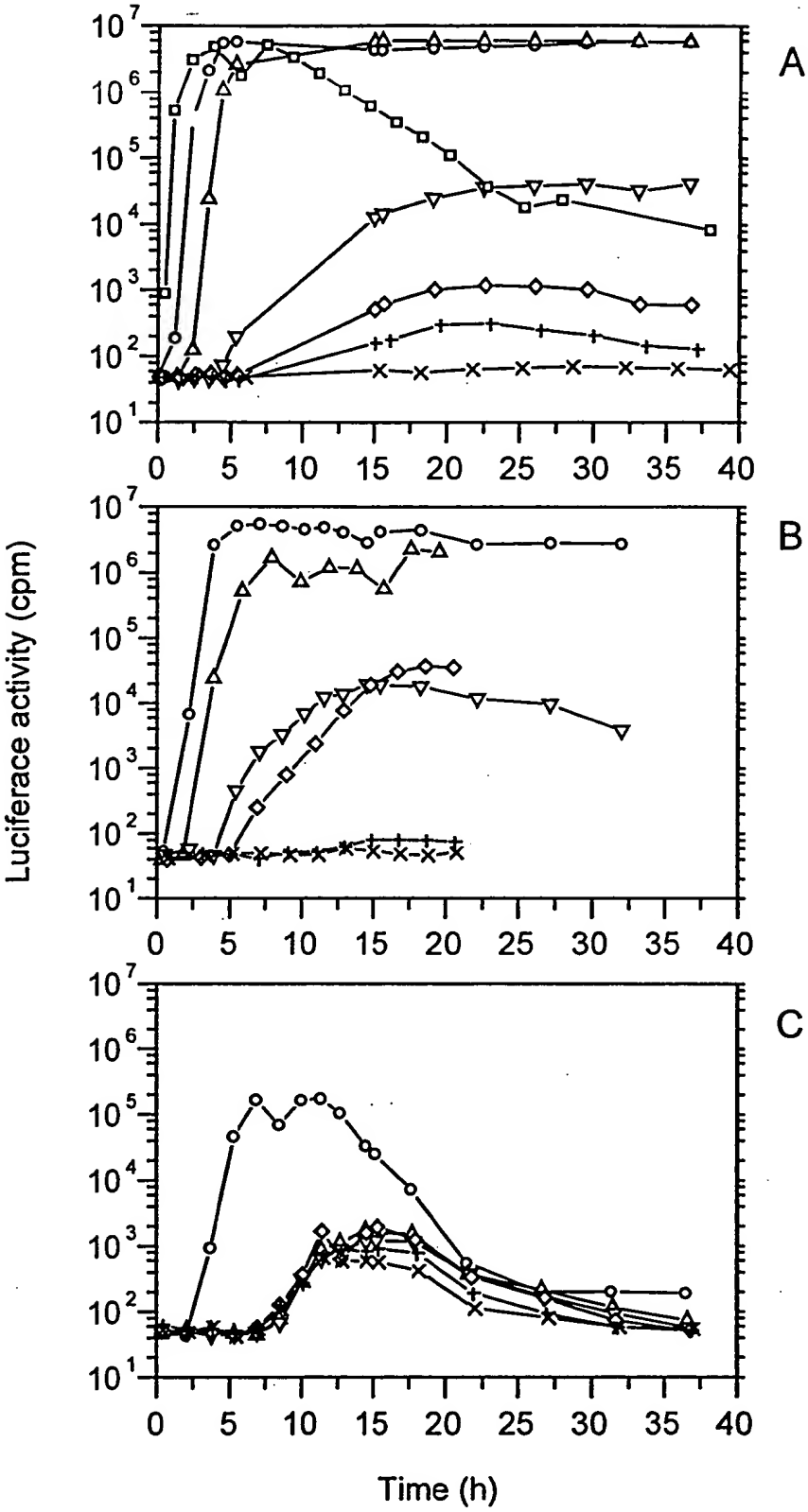
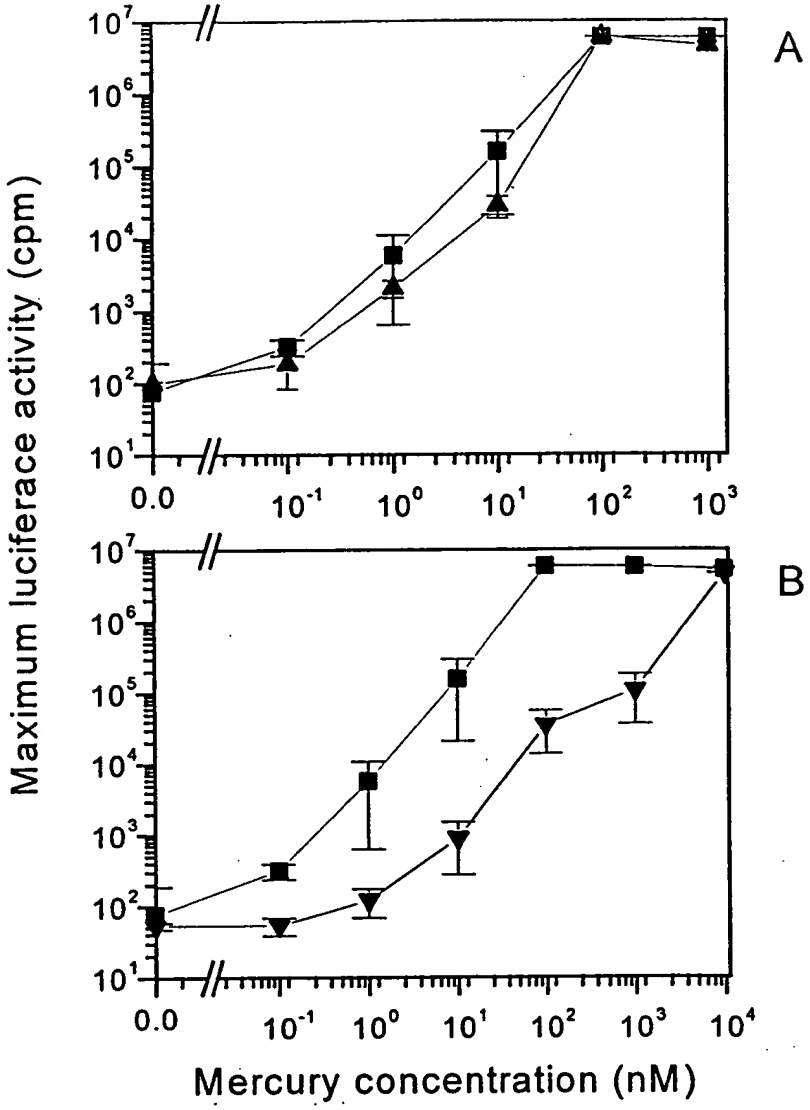

A
B
C
Luciferace activity (cpm)
Time (h)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0 5 10 15 20 25 30 35 40

Fig. 15


Maximum luciferace activity (cpm)
A
B
0.0
Mercury concentration (nM)